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Molecular marker-assisted selection for enhanced yield in malting barley

Deric A. Schmierer[1], Nejdet Kandemir[2], David A. Kudrna[3], Berne L. Jones[4], Steven E. Ullrich[1] and Andris Kleinhofs[1,5,*]

*[1]Dept. of Crop & Soil Sciences, Washington State University, Pullman, WA 99164-6420, USA; [2]Gaziosmanpasa Universitesi, Ziraat Fakultesi, Tarla Bitkileri Bolumu, 60110, Tokat, Turkey; [3]Arizona Genomics Institute, Dept. of Plant Sciences, University of Arizona, Tucson, AZ 85721-0036, USA; [4]Cereal Crops Research Unit, USDA-ARS, Madison, WI 53705-2334, USA; [5]School of Molecular Biosciences, Washington State University, Pullman, WA 99164-4660, USA; *Author for correspondence (phone: (509) 335-4389; fax : (509) 335-8674; e-mail : andyk@wsu.edu)*

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Abstract

Brewers are reluctant to change malting barley (*Hordeum vulgare* ssp. *vulgare* L.) cultivars due to concerns of altered flavor and brewing procedures. The U.S. Pacific Northwest is capable of producing high yielding, high quality malting barley but lacks adapted cultivars with desirable malting characteristics. Our goal was to develop high yielding near isogenic lines that maintain traditional malting quality characteristics by transferring quantitative trait loci (QTL) associated with yield, via molecular marker-assisted backcrossing, from the high yielding cv. Baronesse to the North American two-row malting barley industry standard cv. Harrington. For transfer, we targeted Baronesse chromosome 2HL and 3HL fragments presumed to contain QTL that affect yield. Analysis of genotype and yield data suggests that QTL reside at two regions, one on 2HL (ABG461C-MWG699) and one on 3HL (MWG571A-MWG961). Genotype and yield data indicate that additional Baronesse genome regions are probably involved, but need to be more precisely defined. Based on yield trials conducted over 22 environments and malting analyses from 6 environments, we selected one isogenic line (00-170) that has consistently produced yields equal to Baronesse while maintaining a Harrington-like malting quality profile. We conclude there is sufficient data to warrant experiments testing whether the 2HL and 3HL Baronesse QTL would be effective in increasing the yield of other low yielding barley cultivars.

Abbreviations: BG – β-glucan; cv – cultivar; DP – diastatic power; ME – malt extract; PSN – Preliminary State Nursery; SP – soluble protein; S/T – soluble/total protein; SUN – Washington Spring Barley State Uniform Nursery

Introduction

Investigation and manipulation of quantitative traits is a very difficult and imprecise task when compared to simple qualitative traits. Many traits of agronomic importance are inherited quantitatively, complicating cultivar improvement (Stuber 1994). Independent manipulation of the numerous loci contributing to quantitative traits is confounded by the fact that some loci may contribute little genetically relative to the overall phenotypic expression (Thoday 1961). Continuous distribution of phenotypes makes it difficult to select progeny of hybrids with desired attributes and to correctly characterize genotypic groups without the aid of markers. Environmental effects on performance across generations, years, and locations,

and interactions among quantitative trait loci (QTL) also make correct analysis a difficult task (Paterson et al. 1991; Hayes et al. 1993). As a result, large populations, extensive replication, and complex statistical analyses must be used to detect and select for the loci presumably controlling quantitative traits.

Moderately dense genetic maps have been constructed for many crop species, including barley (Graner et al. 1991; Kleinhofs et al. 1993; Kasha et al. 1995; Costa et al. 2001). These maps have led to increased accuracy of locating QTL to specific chromosomal regions. Genotypes of quantitative loci of interest, and their location relative to marker loci, can be inferred from their phenotypic means and variances of the progeny of a test cross, as well as the genotypes of marker loci (Thoday 1961). The use of molecular markers allows for the transfer of desired QTL while eliminating genome regions with negative effects. Based on a map density of markers spaced at 20cM intervals, there is less than a 1% chance of double crossovers occurring between flanking markers with the same genotype (Lander and Botstein 1989).

Several key strategies have been used to employ molecular marker-assisted selection (MAS) in crop improvement. One involves using QTL information to design matings that will maximize the probability of pyramiding desired QTL in a single genotype (Dudley 1993; Hayes et al. 1996; Zhu et al. 1999). Another approach involves the introgression of a limited number of QTL via marker-assisted backcrossing (Dudley 1993). A method derived from the backcross scheme, but adapted to introgress exotic germplasm, was designated advanced backcross QTL (AB-QTL) analysis (Tanksley and Nelson 1996). This strategy was developed to integrate QTL studies with variety improvement. AB-QTL analysis was used to introgress wild tomato QTL alleles into elite cultivated tomato to improve several agronomically important traits (Bernacchi et al. 1998). Near-isogenic lines (NILs) were developed that showed an improvement over the control for seven traits. Studies such as these have lead to an increased accuracy of localization and eventual cloning of genes controlling quantitative traits (Frary et al. 2000; Takahasi et al. 2001).

Experiments involving MAS for grain yield have been conducted in barley. Both molecular and phenotypic selection strategies were used to assess the validity of QTL in a population of 92 'Steptoe' x 'Morex' (SM) doubled haploid lines (DHLs) not used in the original mapping population (Romagosa et al. 1999). MAS for alleles contributed by Steptoe and Morex on chromosomes 3H and 6H, respectively, significantly increased grain yield. This result confirmed a previous report by Romagosa et al. (1996) that these QTL were associated with increased yield. Zhu et al. (1999) attempted to cross SM DHLs to pyramid QTL for yield. They found that due to epistasis and QTL x environment interactions, genotypes with putatively favorable alleles did not always perform as expected. Three grain yield QTL on chromosomes 2HS, 3H, and 1H(5)L were investigated via MAS backcross breeding (Kandemir et al. 2000a, b). While yield related traits such as extended flowering time (2HS QTL) and reduced height, lodging, and head shattering (3H QTL) were successfully transferred (Kandemir et al. 2000b), significant yield increases were not achieved (Kandemir et al. 2000a). These results demonstrate the difficulty in using MAS to realize yield increase.

Here we report on experiments to transfer QTL associated with yield from the cv. Baronesse, a high yielding feed barley, to the cv. Harrington, the North American malting industry two-row standard. Baronesse QTL were genetically mapped to the long arms of barley chromosomes 2HL and 3HL in a Lewis x Baronesse population primarily using AFLP markers (T. Blake, person. comm.). We developed NILs for 2HL and 3HL using RFLPs via a backcross breeding scheme. The intention was to create high yielding lines that maintain a high quality malting profile and to verify and refine the location of the previously suggested Baronesse QTL.

Materials and methods

Development of NILs

Baronesse (donor) pollen was applied to florets of two separate Harrington (recipient) plants in 1997 for use in NIL development. Eight F_1 seeds were collected from each Harrington plant and planted in the greenhouse. The 16 F_1 progeny were backcrossed to the recurrent parent Harrington to produce 279 BC_1F_1 progeny, which were grown in the greenhouse in Pullman, WA in 1998. For convenience, only 200 of the BC_1F_1 progeny were genotyped. Sixteen plants containing Baronesse alleles at target regions on chromosomes 2HL and 3HL were selected based on RFLP marker genotypes and backcrossed to the recurrent parent. One hundred forty-four BC_2F_1

progeny were obtained which were also grown in the greenhouse later in 1998. Seventeen progeny were selected based on genotypes in the 2HL and 3HL region and allowed to self-pollinate to the BC_2F_2. Nine of the 17 BC_2F_1 progeny containing large, overlapping Baronesse fragments (~20-60cM) of 2HL and 3HL were selected and backcrossed to Harrington to produce 63 BC_3F_1 progeny. The BC_2 families, 106 in total, were sent to Southern Seed Technology in Leeston, New Zealand for seed increase in October of 1998. The 63 BC_3F_1's were allowed to self-pollinate. Eleven individual plants were selected based on genotypes in the 2HL and 3HL regions. Individual seeds were collected from the 11 BC_3F_1's and planted in the greenhouse resulting in 144 BC_3F_2 progeny. These subsequent plants were subjected to genotype analysis and assigned to families. Sixty-five of the BC_3F_2 families were selected based on genotypes in the 2HL and 3HL regions. These families were advanced to the F_3 where individual plants were selected based on the most desirable genotypes. Several individual heads were randomly selected from each family to account for variation in unselected genome regions resulting in 144 BC_3F_3 lines. These were planted in the field at Pullman in 1999 in single, unreplicated head-rows. One hundred-forty single BC_3F_3 plants were selected based on F_2 genotypic data and sent to New Zealand in October 1999 for seed increase.

Agronomic evaluation

Four nurseries were planted in summer 2000 at Spillman Farm at Pullman, WA. Plot size harvest area was 1.5m × 1.8m. Nursery 1 consisted of 28 of the highest yielding BC_2 lines from a preliminary trial planted in Pullman in 1999 plus Harrington and Baronesse (data not shown). Each BC_2 line was genetically unique as determined by molecular markers, but not necessarily homozygous or homogeneous. Nurseries 2-4 each contained the parents and 28 BC_3F_5 lines selected for homozygosity on chromosomes 2HL and 3HL for a total of 84 BC_3 independent entries.

In summer 2001, 28 lines were planted in a preliminary state nursery (PSN) at three locations, Pullman, WA, Fairfield, WA, and Royal Slope, WA. Plot size harvested for all locations was 1.5m x 6.1m. This nursery consisted of 8 BC_2 lines, 20 BC_3 lines, and the parents. These lines were selected to represent the highest yielding lines from both 1999 and 2000 with the exception of 00-175, which was included as a low yielding check.

Five lines consistently producing Baronesse-like yield and Harrington-like malting quality were identified and planted in the Washington Spring Barley State Uniform Nursery (SUN) at 15 locations in 2002 (http://variety.wsu.edu/). Two additional high yielding lines were planted in a PSN in 2002 at three locations, Pullman, WA, Ritzville, WA, and Royal Slope, WA. Seeding and fertilizer rates consistent with local farming practices were applied to all yield trials. A randomized complete block experimental design with three replications was used for all nurseries. Statistical analysis was performed using SAS proc GLM function (SAS Institute 1988).

Malting quality analysis

Malting quality analysis was performed on two replicates of 62 randomly selected lines plus the parents grown at Pullman in summer 2000. To obtain the highest malting potential, seed from the Royal Slope irrigated nursery was analyzed in 2001 and 2002. Analyses were conducted on 3 replicates in 2001 with the exception of 00-117, where seed for only 2 replicates were available. One replicate was tested in 2002 due to quantity limitations, precluding any statistical analysis. Malting was performed at the USDA-ARS Cereal Crops Research Unit at Madison, WI using 170 g samples in a micro-malting system. Standard malting procedures were followed as described by the American Society of Brewing Chemists (1992). Statistical analysis was performed using SAS proc GLM function (SAS Institute 1988).

Genetic analysis

DNA extraction and hybridization for RFLPs were modified from Kleinhofs et al. (1993). When dry leaf tissue was not available, 1.3g of fresh leaf tissue from 1- to 2-week-old seedlings was used for extraction. Digested DNA was electrophoresed on 1% agarose gels. Hybridization for non-barley probes was done overnight at ~60 ^{o}C. Membranes were exposed to X-ray film for 1–7 days depending on hybridization intensity using NEN Reflection QF intensifying screens. Once polymorphism was established (Table 1), the progeny were then genotyped. The genomic backgrounds of all lines were screened with RFLPs at an interval of approximately 10–20cM (Figure 1).

Table 1. Chromosome location and enzyme polymorphisms for RFLPs used to genotype Harrington/Baronesse NILs.

Probe	Chromosome	Enzyme used to genotype
RFLPs used to genotype 2HL and 3HL target QTL regions		
MWG520	2 (2H)	EcoRV
ABG005	2 (2H)	DraI
ABG461C	2 (2H)	EcoRI & HindIII
MWG699	2 (2H)	XbaI
MWG865	2 (2H)	EcoRV
Ole2	2 (2H)	EcoRV
ABG1032	2 (2H)	XbaI
ABG072	2 (2H)	XbaI
CDO244	2 (2H)	XbaI
ksuD22	2 (2H)	EcoRV
ABC252	2 (2H)	EcoRV
ABC157	2 (2H)	XbaI
MWG660	2 (2H)	HindIII
MWG571	3 (3H)	EcoRI
MWG961	3 (3H)	HindIII
WG110	3 (3H)	EcoRV
ABC805	3 (3H)	HindIII
MWG041	3 (3H)	XbaI
RFLPs used to scan the genome of each NIL		
ABG704	1 (7H)	EcoRV
ABC327	1 (7H)	BamHI
BE214786	1 (7H)	HindIII
ABG380	1 (7H)	DraI
ABC253	1 (7H)	HindIII
ABG608	1 (7H)	XbaI
ABG058	2 (2H)	BamHI
DAK605	2 (2H)	EcoRI
BCD1532	3 (3H)	EcoRI
BCD706	3 (3H)	HindIII
ABG396	3 (3H)	XbaI
PSR156A	3 (3H)	HindIII
MWG634	4 (4H)	DraI
BF258346	4 (4H)	HindIII
MWG058	4 (4H)	DraI & HindIII
BG416882	4 (4H)	BamHI & XbaI
BG417996	4 (4H)	XbaI
ABG601	4 (4H)	DraI
MWG938	5 (1H)	BamHI
BCD098	5 (1H)	EcoRV
BF065140A	5 (1H)	HindIII
BG367156	5 (1H)	DraI
Cxp3	6 (6H)	EcoRV
ABG020	6 (6H)	XbaI
MWG820	6 (6H)	BamHI
MWG934	6 (6H)	BamHI
MWG897	6 (6H)	HindIII
Gsp	7 (5H)	BamHI
BE602168	7 (5H)	EcoRI
BE195592	7 (5H)	BamHI
ABC717	7 (5H)	BamHI
BF623192	7 (5H)	XbaI
ABC622	7 (5H)	DraI & XbaI
ABC718	7 (5H)	XbaI

Substitution mapping was used to refine the QTL regions (Paterson et al. 1990; Han et al. 1997; Han et al. 1999). By comparing genotypes of progeny to phenotypic data and selecting NILs with overlapping fragments of DNA containing a QTL, the interval between flanking markers was reduced to a relatively narrow margin.

Results

Analysis of QTL associated with yield

NILs selected based on their chromosome 2HL and 3HL genotypes that showed promise in preliminary yield trials were further analyzed to verify and refine Baronesse chromosome fragments that might affect yield. Lines 00-106 to 111 yielded comparable to Baronesse in the 2000 Pullman preliminary yield trials (Table 2, Table 3) and shared the Baronesse chromosome 2H segment maximally from ABG461C to Ole2 (Figure 2). Lines 00-124 and 126 and NZDK102, 103, and 106 yielded significantly less than Baronesse in 2000 and 1999 Pullman preliminary trials, respectively (Table 3), and shared the Baronesse chromosome 2H MWG699 to Ole2 segment (Figure 2). These data suggested that a chromosome 2H QTL resides in the ABG461C to MWG699 region (Figure 2). The suggested chromosome 3H QTL was established by the lines 00-146, 00-165, and 00-170, which yielded comparable to Baronesse in the 2000 Pullman preliminary trials and the 2001 PSN at three locations (Table 2). These lines shared the Baronesse chromosome 3H MWG571A to MWG961 segment identifying it as a QTL affecting yield (Figure 2). Line 00-123 yielded similar to Baronesse in the 2000 Pullman preliminary trials and the 2001 PSN at three locations (Table 2). This line contains the chromosome 3H QTL, but not the chromosome 2H QTL (Figure 2). However, it does contain two additional chromosome 2H fragments from ABG072 to ksuD22 and ABC252 to MWG660 (Figure 2), suggesting additional QTL affecting yield on chromosome 2H. This data suggests that our analysis has refined the QTL regions associated with yield from the original analysis (T. Blake, person. comm.), which broadly localized the QTL to the entire chromosome arms of 2HL and 3HL.

An additional NIL, NZDK7, consistently produced grain yields comparable to Baronesse (Table 2), but did not contain detected Baronesse alleles in the 2HL

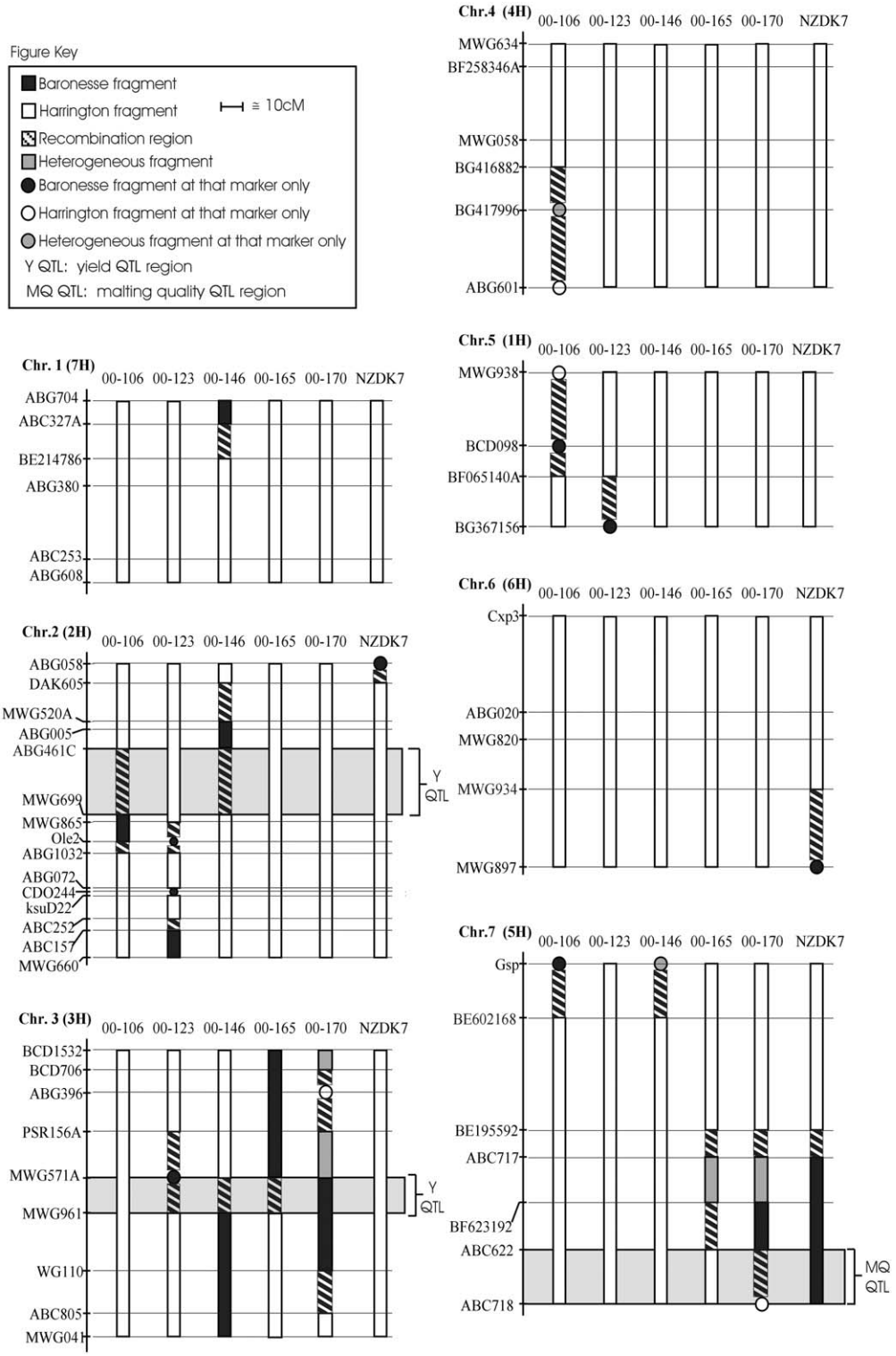

Figure 1. Genotypes of six Harrington/Baronesse NILs for all seven barley chromosomes. A column represents the chromosome of each NIL. A line number at the tip of each column designates individual NILs. Maps represent each barley chromosome, with the short arm towards the top of the Figure and the long arm towards the bottom. Horizontal lines extending from each marker represent that marker's genetic position for a particular NIL. The color scheme indicates the genotype of each NIL and is outlined in the Figure key. Shaded areas on 2H, 3H, and 5H indicate QTL regions of interest.

Table 2. Grain yields (kg ha^{-1}) of Harrington/Baronesse NILs with Baronesse-like yield and Harrington-like malting quality, along with Baronesse-like yielding line NZDK7. Data was taken from Pullman, WA in 2000, PSN[a] in 2001 and 2002, and SUN[b] in 2002. Grain yield for each line is equal to Baronesse unless otherwise noted.

	2000	2001	2002		
Line #	Pullman, WA	PSN	SUN	9 Hi-pro SUN[c]	PSN
Baronesse	6952	6677	4648	5622	5142
00-106	7342	6507	4110*	5031*	–
00-123	7114	6475	4252*	5237*	–
00-146	7073	6579	4104*	5114*	–
00-165	6957	6419	4337*	5262*	–
00-170	6866	6508	4386*	5470	–
NZDK7	6596	6544	–	–	5133
Harrington	6166*	6354	4184*	5182*	5029

[a]Preliminary state nursery at Fairfield, WA, Pullman, WA, and Royal Slope, WA in 2001; Ritzville, WA, Pullman, WA, and Royal Slope in 2002; [b]Washington Spring Barley State Uniform Nursery at 15 locations (see http://variety.wsu.edu/); [c]9 high production environments of the SUN: Dayton, WA, Fairfield, WA, Farmington, WA, Mayview, WA, Pullman, WA, Reardan, WA, Royal Slope, WA, St. John, WA, Walla Walla, WA; *Significantly different than Baronesse at $P \leq 0.05$.

Table 3. Grain yields (kg ha-1) of Harrington/Baronesse NILs not included in Table 2 that helps delimit the putative yield QTL regions. Data was recorded from Pullman, WA in 1999 and 2000 and the PSN[a] in 2001. Grain yield for each line is equal to Baronesse unless otherwise noted.

	Pullman, WA		PSN
Line #	1999	2000	2001
Baronesse	5837	6952	6677
Harrington	4620*	6166*	6354
NZDK102	4525*	–	–
NZDK103	4787*	–	–
NZDK106	4806*	–	–
00–107	–	6727	–
00–108	–	6857	6393
00–109	–	7193	6297
00–110	–	6822	–
00–111	–	7422	6182*
00–124	–	6537*	–
00–126	–	5930*	–
00–137	–	6961	6155*

[a]Preliminary state nursery at Fairfield, WA, Pullman, WA, and Royal Slope, WA; *Significantly different than Baronesse at $P \leq 0.05$.

or 3HL regions (Figure 1). The F_1 parent for this NIL contained a Baronesse fragment on 2HL, but presumably a recombinant was inadvertently selected for further line development that contained the alternative Harrington fragment. These data suggested the existence of additional genes associated with yield.

Yield and genomic background analysis

We selected 186 NILs, based on markers alone in the early generations of selection, for phenotypic analysis. Initially, 109 NILs (or ~58%) yielded similar to Baronesse (Schmierer 2002). Five NILs representing different cv. Baronesse chromosome segments were identified as producing Baronesse-like yield in preliminary trials and were advanced to the SUN yield trials in 2002 (Table 2). All lines had Harrington-like malting quality and contained at least one of the two putative QTL. Averaged across the 15 locations, one line, 00-170, yielded significantly more than Harrington, but less than Baronesse ($P \leq 0.05$). All other lines yielded significantly less than Baronesse and equal to Harrington. Considering only the nine high-production malting barley-growing environments, NIL 00-170 yielded equal to Baronesse and greater than Harrington at $P \leq 0.05$.

The NIL NZDK7 has consistently produced yields comparable to Baronesse in all preliminary trials, but was not included in the 2002 SUN due to poor malting quality. This line again yielded equal to Baronesse in the 2002 PSN (Table 2).

The genomic background of the five NILs advanced to the SUN and NZDK7 was analyzed. The backgrounds were mostly Harrington, but Baronesse genomic segments in addition to the ones on chromosomes 2H and 3H were detected in all lines (Figure 1). There were no Baronesse segments that were common to all lines, thus it was impossible to conclude the potential contributions of these additional segments to the yield performance of the NILs.

Malting quality analysis

Malting quality analyses were performed on samples from 2000 Pullman and from the 2001 Royal Slope nurseries (Schmierer 2002). Analysis and comparison to the ideal commercial malt criteria for breeders (http://www.ambainc.org/ni/index.htm) and to Harrington controls permitted selection of NILs that maintained malting quality similar to Harrington, while demonstrating Baronesse-like yield. Although 00-106 and 00-146 had diastatic power values significantly less than Harrington in 2001 (Table 4), the previous year's performance and remaining characteristics were sufficient to enable these lines to be

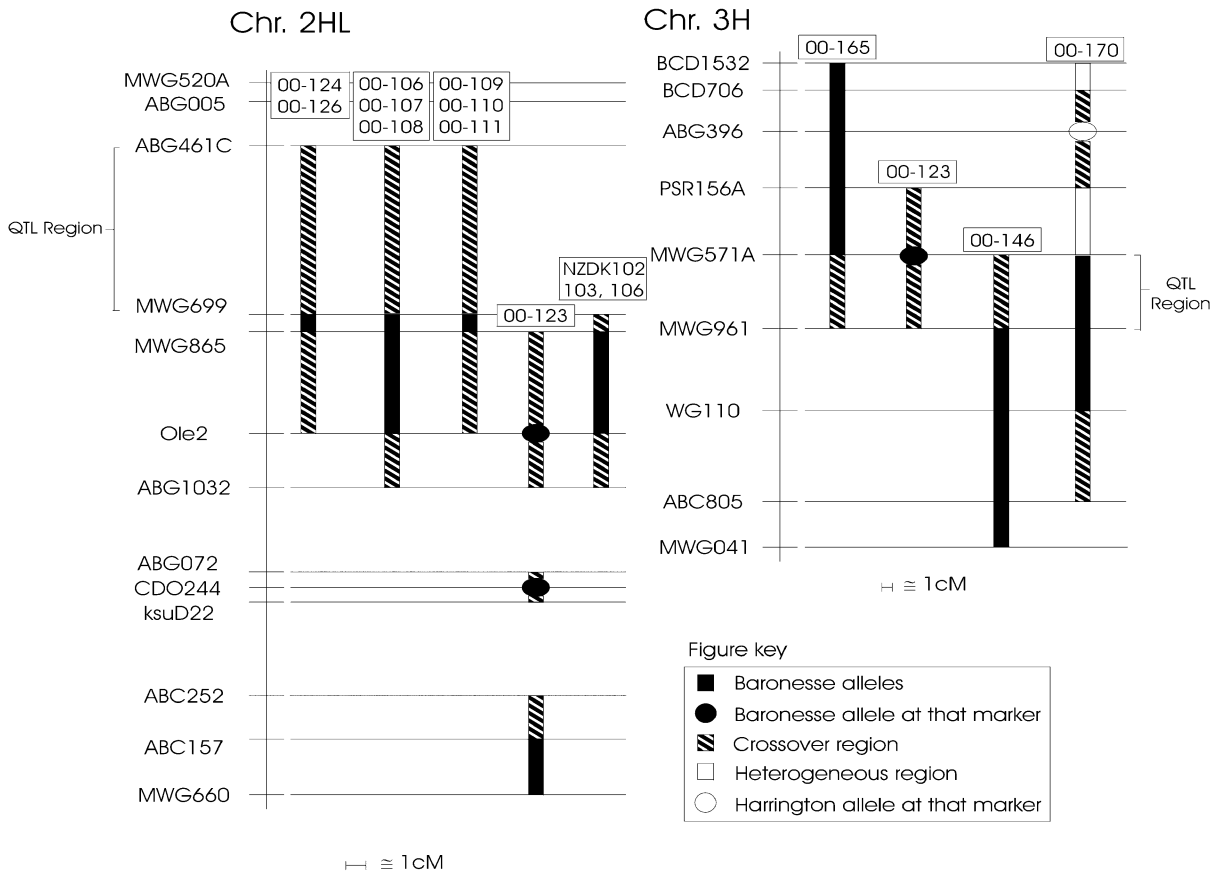

Figure 2. Genotypes of selected Harrington/Baronesse NILs for 2HL and 3HL that delimit each putative QTL region associated with yield. The Baronesse fragment of 2HL and 3HL for selected NILs is represented by a column and designated by a line number at the top of each column. Maps represent the long arm of each chromosome oriented with the centromere on top and the telomere on bottom. Horizontal lines running from each marker name to the right of the Figure indicate that marker's genotype for that selection.

analyzed further. This process allowed the selection of the five NILs advanced to the SUN. These lines, plus NZDK7 were again analyzed for malting quality using seed from the 2002 crop grown in the Royal Slope nursery. All five lines exhibited Harrington-like malting quality at all parameters tested (Table 4). Line NZDK7 showed low malting quality particularly deficient in malt extract and α-amylase activity (Table 4). The β-glucan content was abnormally high and out of the commercial malt range for all lines including Harrington. Since this was abnormal for the high quality cv. Harrington, we concluded that it was most likely due to the irrigated environmental conditions used to grow the crop and not representative of the genotype. The malting quality data were consistent for all lines for all years (for details see Schmierer, 2002).

Discussion

This project was initiated with the goals of 1) developing a high yielding barley with Harrington-like malting quality and 2) analyzing the Baronesse QTL associated with yield. The approach was to transfer fragments of Baronesse chromosome 2HL and 3HL, previously suggested to contain yield associated QTL (T. Blake, person. comm.), to Harrington using a backcross approach facilitated by molecular marker selection. The NILs developed were genotyped and yield tested over several years in order to associate yield performance with molecular genotype. Approximately 58 % of the NILs initially selected via markers yielded equal to Baronesse, which is a greater percentage than expected for phenotypic selection alone. Moreau et al. (1998) reported that one could achieve an increased relative efficiency of MAS over

Table 4. Malting quality performance means of Harrington/Baronesse NILs from trials grown in 2000-2002. All values are equal to Harrington unless otherwise noted. Statistical significance when compared to Harrington is at P ≤ 0.10*; 0.05**; 0.01***.

Line #/ID	Malt Extract (%)	Barley Protein (%)	Soluble Protein (%)	Soluble/Total Protein (%)	Diastatic Power (°ASBC)	α-amylase (20 °DU)	β-glucan (ppm)
Ideal Commercial Malt Criteria	> 81.0 or < Harrington	11.5-13.0 or ≤ Harrington	4.9-5.5 or < Harrington	40-45 or < Harrington	120-140 or ≈ Harrington	45-60 or ≈ Harrington	< 115 or ≤ Harrington
2000[a]							
Baronesse	76.5***	11.8	3.75***	33.6***	89	43.9***	154
Harrington	79.3	11.9	4.73	42.5	97	63.7	144
00-106	79.8	10.4**	4.31***	42.9	89	63.5	240***
00-123	81.3***	10.0***	4.49**	48.7***	80*	71.1***	100
00-146	78.5	12.8	4.61	37.9*	131***	65.7	242***
00-165	–	–	–	–	–	–	–
00-170	–	–	–	–	–	–	–
NZDK7	78.4	11.9	3.95***	34.1***	71***	45.8***	333***
2001[b]							
Baronesse	78.1***	12.9	3.96***	31.5***	73***	42.1***	472
Harrington	80.7	13.6	5.79	45.2	105	70.2	496
00-106	80.8	13.2	5.30**	42.1**	88***	70.1	475
00-123	81.4	13.0	5.65	44.1	97*	75.2**	457
00-146	81.0	13.1	5.44*	43.5	93**	66.0**	574
00-165	81.1	12.3	5.31**	44.8	105	68.4	428
00-170	81.2	13.1	5.64	44.3	109	65.9**	465
NZDK7	78.9***	13.5	4.48***	34.2***	98	47.0***	627***
2002[c]							
Baronesse	79.3	10.8	4.16	40.3	67	44.1	345
Harrington	82.0	11.3	6.26	56.6	89	74.0	404
00-106	82.1	11.4	5.33	49.2	78	72.4	270
00-123	81.8	12.6	5.93	48.9	101	79.5	299
00-146	81.5	12.5	5.59	47.0	117	70.6	424
00-165	81.7	12.7	5.85	48.3	94	82.6	376
00-170	81.5	12.7	5.96	47.4	88	75.7	436
NZDK7[b]	79.5	12.0	4.08	35.1	110	49.5	519

[a]Malting samples were taken from the dryland Pullman, WA nursery; [b]Malting samples were taken from the irrigated Royal Slope, WA nursery from the PSN; [c]Malting samples were taken from the irrigated Royal Slope, WA nursery from the SUN.

phenotypic selection for traits with low heritabilities ($h^2 = 0.234$ in 2001) and a finite population size ($\geq$ 100). Finally, the NILs were also tested for malting performance. From these analyses, several lines emerged that incorporated Baronesse-like yield and maintained a Harrington-like malting profile. Based on the 2002 nine site yield trials, NIL 00-170 produced Baronesse-like yield and Harrington-like malting quality. This NIL has been subjected to further tests and is being considered for variety release. Thus, goal #1 was at least partially achieved.

Yield data and genotype analysis suggested that we have verified and refined the chromosome 2H and 3H QTL regions. The chromosome 3H QTL was the most stable in terms of it's effect on yield. It is present in 00-170, the only line with Baronesse-like yield in 2002, and in 00-123, 146, and 165. All of the chromosome 3H QTL lines have other Baronesse chromosome segments in their background, but there is no commonality among them. The possible exceptions are lines 00-165 and 170, which have a chromosome 5H(7) fragment in common with each other and with NZDK7. This genomic region deserves further analysis. The evidence for the chromosome 2H QTL is weak. Only line 00-106 (and its family members) has this genome region from Baronesse. While the performance of this line was excellent in the preliminary trials, it was identical to Harrington in the 2002 extensive yield trials. Interestingly, this 2H chromosomal region contains the *vrs1* locus that controls spike-type in barley. This locus has been shown to be associated with a number of agronomic and yield related traits in previous QTL studies (Kjaer and Jensen 1996; Marquez-Cedillo et al. 2001; Dahleen et al. 2003; Mesfin et al. 2003).

QTL x environment interactions (QTL x E) could account for why several of the NILs displayed a significant reduction in yield compared to Baronesse in 2002. QTL x E have been reported to effect yield in barley DHLs (Hayes et al. 1993). Associations of QTL x E were shown even in chromosomal regions with no significant average QTL effect. Analysis of the 2002 data from the locations used for the 2001 PSN revealed that only lines 00-106 and 00-146 yielded significantly less than Baronesse (data not shown). This suggests that 00-170 contains Baronesse genes that allow it to adapt to more diverse environmental conditions. NIL 00-170 contains two potentially unique Baronesse fragments (3HL, 5HL) that would be suspected to contribute to this effect. An early spring frost at several locations, plus dryer conditions during seeding and early plant establishment in all rainfall zones (see http://variety.wsu.edu/) are other examples of environmental instabilities not selected for in previous years that could have influenced the 2002 data. None of the additional Baronesse genomic regions found in the five NILs selected for advanced testing affected their malting quality in a negative way.

Line NZDK7 had no detectable Baronesse fragments from the chromosome 2H and 3H QTL regions. Small fragments that may have gone undetected can not be excluded from consideration. Nevertheless, NZDK7 has demonstrated Baronesse-like yield in three years of yield trials. It does contain small Baronesse fragments at the 2HS telomere and 6HL telomere as well as a substantial portion of chromosome 5H(7) long arm. These fragments must be considered as harboring potential QTL affecting yield and negative malting quality genes. Zhu et al. (1999) and Kandemir et al. (2000a) also found that genome regions other than the major QTL targeted for selection may enhance yield expression. A portion of chromosome 5H(7) is shared by NZDK7, 00-170, and 165, which lends support for this region having QTL affecting yield.

In spite of having a mostly Harrington genome, line NZDK7 has poor malting quality as demonstrated by a low ME, SP, S/T, and DP as well as a high BG. QTL for all of these traits have been mapped to the telomeric region of 5H(7)L (Mather et al. 1997; Igartua et al. 2000). These QTL were mapped in a Harrington x TR306 DHL population, with Harrington contributing the favorable alleles. NZDK7 contains Baronesse alleles in this region, possibly explaining the low malting quality of this line.

The presence of epistatic interactions and QTL x E between target QTL and Baronesse fragments remaining in the genome need to be confirmed. Gene action and positive and/or negative allele effects should also be determined. We feel there is sufficient data to warrant experiments testing whether the 2HL and 3HL Baronesse QTL would be effective in increasing the yield of other low yielding barley cultivars, and these experiments are underway. In an attempt to achieve yields comparable to, or even surpass Baronesse across more environments, experiments are being conducted to combine the 2HL and 3HL QTL into a single genotype. Experiments are also being conducted to determine which regions of the genome outside 2HL and 3HL may contribute to yield, with

portions of 2HS, 6HL, and 5HL from NZDK7 being leading candidates.

In summary, using molecular markers to select for specific chromosome regions with potential positive yield contributions enhanced the breeding success for high yield while maintaining traditional malting quality. The identification of specific yield QTL however is problematic due to gene interactions and large variations in yield between replicates and years.

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